PATENT
Atty. Docket No. LEX-003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Gillies *et al.*	CONFIRMATION NO.:	9492
SERIAL NUMBER:	09/256,156	GROUP ART UNIT:	1647
FILING DATE:	February 24, 1999	EXAMINER:	Kapust, R.B.
TITLE:	Enhancing the Circulating Half Life of Antibody-Based Fusion Proteins		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION BY STEPHEN GILLIES AND KIN-MING LO UNDER 37 C.F.R. 1.131

Sir:

We, Stephen Gillies and Kin-Ming Lo, hereby declare as follows:

1. We have reviewed claims 1, 3, 6-8, 10, 29 and 30 as submitted herewith. We are the co-inventors of the subject matter of claims 1, 3, 6-8, 10, 29 and 30 of the above-identified patent application.

2. We make this declaration to establish invention of the subject matter of claims 1, 3, 6-8, 10, 29 and 30 prior to the effective date of International Publication No. WO 97/43316, entitled "Physiologically Active Molecules with Extended Half-Lives and Methods of Using Same," which we understand was published on November 20, 1997.

3. Prior to November 20, 1997, we conceived of a gene construct encoding an IL-2 fusion protein, which included an IgG1 hinge region, an IgG1 CH2 domain with a mutation or a deletion that reduced the binding affinity for an Fc receptor, an IgG1 CH3 domain, and an IL-2 moiety. This construct included a region as claimed in claims 1, 3, 6-8, 10, 29 and 30.

4. We attach as the first page of Exhibit 1 a true copy of laboratory notebook page 2 of Kin-Ming Lo, signed by Kin-Ming Lo, except that dates on the page prior to November 20, 1997, have been removed. We attached as a second page of Exhibit 1 a true copy of laboratory log book page 143, except that the dates on the page, which are all prior to November 20, 1997, have been removed. Exhibit 1 demonstrates that we conceived of the invention of claims 1, 3, 6-8, 10, 29, and 30 prior to November 20, 1997.

5. The laboratory notebook page 2 of Kin-Ming Lo shows a schematic representation of a process for introducing mutations and a deletion into the IgG1 CH2 nucleic acid sequence. As indicated on the top of the page, the purpose of this process is to "mutate the FcRγ binding site of γ_1 to decrease binding" to the Fc receptor. The first step of the process is shown in the upper portion of the page and is labeled "1st set PCR" on the left side of the page. The PCR primers were designed to replace 12 nucleotides of the Cγ_1 CH2 sequence with 9 nucleotides of the Cγ_2 CH2 sequence, thereby modifying the encoded protein to have a reduced affinity for the FcRγ receptor. The primers are represented as solid lines on the page, and are identified as 415S, 437A, 1018A, and 1017S. The nucleic acid sequences of primers 1017S and 1018A are provided on laboratory log book page 143. The 1018A and 1017A primers changed the encoded amino acid sequence from Pro_{232}-Glu_{233}-Leu_{234}-Leu_{235}-Gly_{236}-Gly_{237} to Pro_{232}-Pro_{233}-Val_{234}-Ala_{235}-Gly_{236}.

6. The products of the first PCR step were used in a second step, shown in the center portion of laboratory notebook page 2 of Kin-Ming Lo and labeled "2nd set PCR" on the left side of the page. The PCR product was to be cloned into a TA plasmid vector.

7. The bottom portion of laboratory notebook page 2 of Kin-Ming Lo shows a process for constructing a vector containing an IgG1 hinge region, the mutated IgG1 CH2 domain with reduced binding affinity for an Fc receptor, an IgG1 CH3 domain, and an IL-2 moiety. The cloning strategy included ligating a 224 base pair (bp) fragment and a 1100 bp fragment, producing a 1324 bp fragment as shown on the bottom-right portion of the page (the

"1324 bp fragment"). The 224 bp fragment was a Pst I-BspH1 restriction fragment encoding an IgG1 hinge region and a mutated N-terminal portion of $C\gamma_1$ CH2. This fragment was to be isolated from the TA vector described above. The 1100 bp fragment was a BspH1-Xho I restriction fragment encoding the remaining portion of $C\gamma_1$ CH2, all of CH3, and the fused IL-2 gene sequence. Thus, the 1324 bp fragment included a region as claimed in claims 1, 3, 6-8, 10, 29, and 30.

8. On or before December 8, 1997, Jie Chen, a technician working under our direction, prepared in the United States a construct including the 1324 bp fragment. We attach as Exhibit 2 true copies of laboratory notebook pages 3, 7, 8, 10, 15, 16, 17 and 18 of Jie Chen, except that the date on laboratory notebook page 3 prior to November 20, 1997, has been removed. Jie Chen signed her laboratory notebook pages 3, 7, 8, 15, 16, 17, and 18. This exhibit is to establish diligence from prior to November 20, 1997, to reduction to practice in the United States of the invention of claims 1, 3, 6-8, 10, 29, and 30.

9. Laboratory notebook page 3 of Jie Chen, dated prior to November 20, 1997, shows a restriction enzyme digest identifying recombinant TA plasmid vectors that include the correctly assembled PCR product described in paragraph 6 of this declaration. The PCR product included an IgG1 hinge region and a mutated N-terminal portion of $C\gamma_1$ CH2.

10. Laboratory notebook page 7 of Jie Chen, dated November 19, 1997, shows that a DNA sequencing reaction was performed to confirm the sequences of the PCR products in the TA vector in isolates ("clones") labeled "2" and "3."

11. Laboratory notebook page 8 of Jie Chen, dated November 20, 1997, shows that DNA sequencing reactions were performed to confirm the sequences of the PCR products in the TA vector in clones labeled "1," "4," "5," and "7."

12. Laboratory notebook page 10 of Jie Chen indicates that the DNA sequence of clone 5 was determined to be correct. This page also shows a process for preparing the 1324 bp fragment described above using the PCR product from clone 5. The top of the page is dated Friday, November 21, 1997. Because the bottom of the page is undated, we do not know when this page was completed, although it would have been completed no earlier than November 21, 1997.

13. Laboratory notebook page 15 of Jie Chen, dated Monday, December 2, 1997, shows another process for preparing the 1324 bp fragment, using the Drd I restriction enzyme instead of BspH1. The BspH1 restriction enzyme is sensitive to the methylation status of the DNA, limiting the usefulness of the enzyme. The Drd I enzyme is not believed to share this limitation. Thus, between Friday, November 21, 1997, and Monday, December 2, 1997, inclusive, a period that included the Thanksgiving holiday, work was done toward preparation of the 1324 bp fragment; a second, more efficient process for preparing the 1324 bp fragment was identified and planned; and restriction fragments were prepared and analyzed in accordance with the improved process. The photographs on the page show the gel-separated restriction fragments used for preparing the 1324 bp ligation product.

14. Laboratory notebook pages 16, 17, and 18, of Jie Chen, dated December 3, 4, and 8, 1997, respectively, detail DNA preparations, ligation reactions, transformations, and restriction digests used to prepare and analyze the 1324 bp ligation fragment. On these pages, the fragment encoding the mutant $C\gamma_1$ CH2 domain was erroneously referred to as "γ_4." Page 18 indicates that a plasmid containing the 1324 bp fragment was successfully prepared on or before Monday, December 8, 1997.

15. All statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true. These statements are made with the knowledge that willful false statements and the like, so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such

willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: January 12, 2004 _____
Stephen Gillies

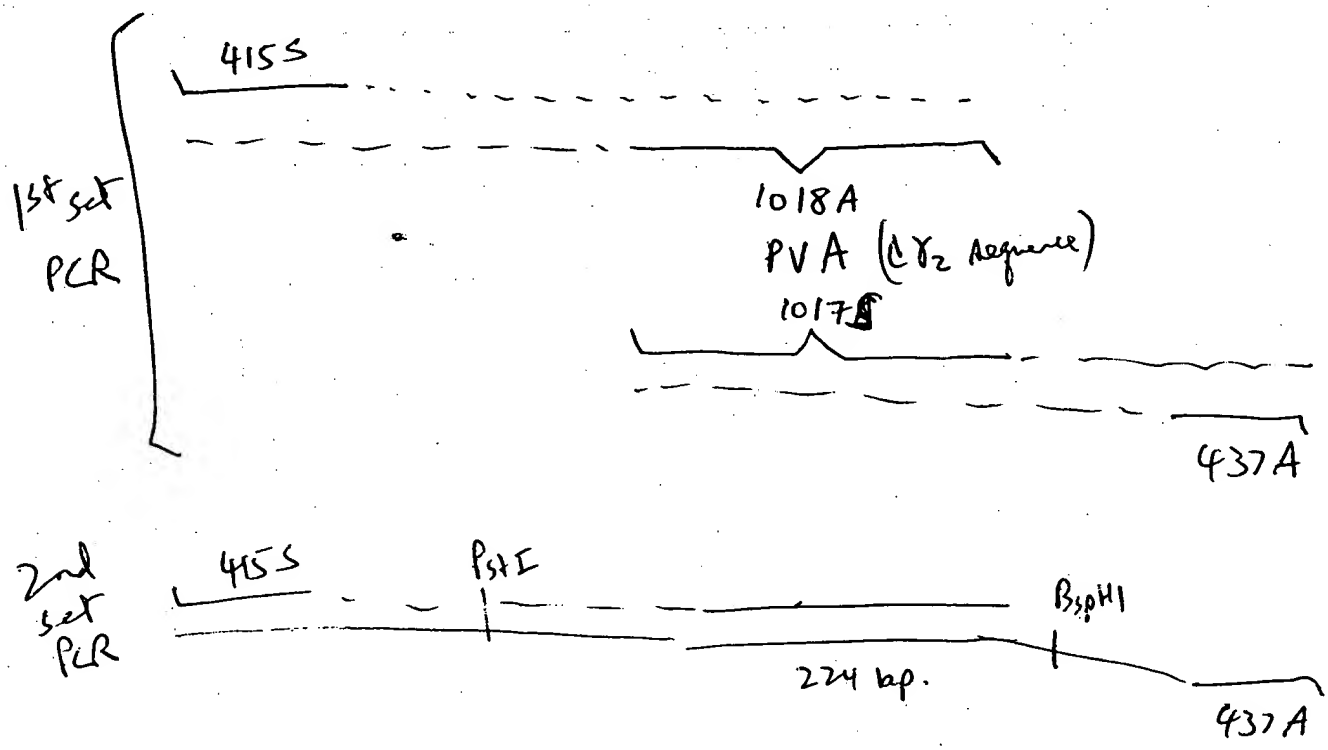
Date: Jan 12, 2004 _____
Kin-Ming Lo

3004201

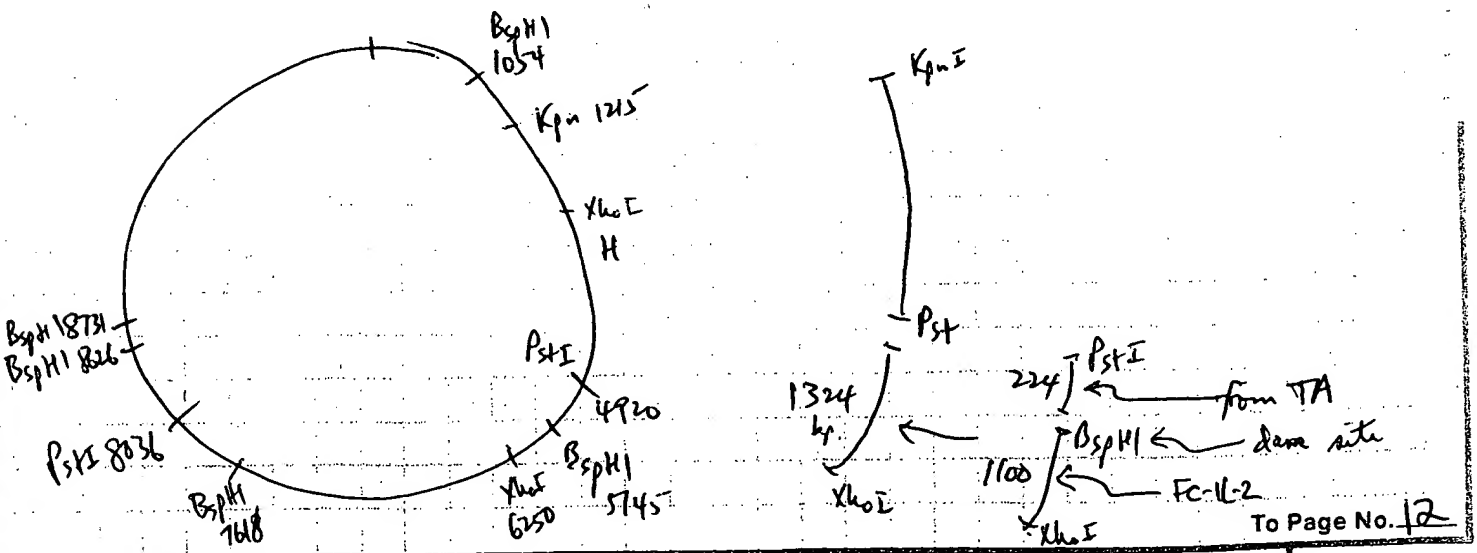
Project No._____
B ok No._____ TITLE pdHL7 - hu KS mutated γ_1 - IL-2

m Page No.__

We want to mutate the FcRγ binding site of γ_1 to decrease binding.



Jie got the PCR product and clone the fragments into TA vector.



To Page No. 12

Witnessed & Understood by me,	Date	Invented by	Date
	12/31/97	Record d by	

Page No.

Cγ1 delete FcR binding

PCR mutagenic primer @ 1070

1017S (022129-1)

36 mer
5'-GCA CCT CCC GTC GCA GGA CCG TCA GTC
TTC CTC TTC

1 OD = 34.6 µg = 3196 pmol
MW = 10824.11 E260 = 312.9 L/mmol-cm
Tm = 78.5 °C ([Oligo]=250 pM; [Salt]=50 mM)

Yield = 22.6 OD; Desalted

AMITOF (617)782-9242 • 1-(800)998-4863 • (617) 782-9352(FAX)

Cγ1 delete FcR binding

PCR mutagenic primer @ 1070

1018A (022129-2)

35 mer
5'-CGG TCC TGC GAC GGG AGG TGC TGA GGA
AGA GAT GG

1 OD = 31.6 µg = 2888 pmol
MW = 10948.25 E260 = 346.3 L/mmol-cm
Tm = 79.7 °C ([Oligo]=250 pM; [Salt]=50 mM)

Yield = 21.9 OD; Desalted

AMITOF (617)782-9242 • 1-(800)998-4863 • (617) 782-9352(FAX)

10195

essed & Understood by me,	Date	Invented by	Date

From Pag No.___

- Mini prep

(pdHL7-huks-IL2) / 4158-487A in TA #1-14

Cut with EcoR.I

#1, 2, 3, 4. 5, 7 are correct.

= Ligation. (in TA)

cDNA : Lox

PCR product : P2 fragment (primer F715-R1486)

≡ Transformation

Lox (P2 fragment) in TA

To Page No.____

Witnessed & Understood by m ,	Date	Inv nted by	Date
Sung Kong		Record d by Jkchen	

From Page N .—

11/19/97

-. Sequencing Reaction.

DNA : hukg in TA (11/14/97)

2, 3

primer : 1019 S , 1020 A

=. muFc - Elisa (repeat test of 11/17/97)

Optical Density

muFc-gHV in CHO | gp120 in CHO

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.291	0.210	0.157	0.364	0.444	0.503	0.184	0.180	0.212	0.825	0.216	0.276
B	1.219	1.316	0.214	0.229	0.480	0.498	0.170	0.178	0.665	0.372	0.219	0.276
C	1.076	1.194	0.209	0.190	0.482	0.469	0.168	0.170	0.913	0.460	0.219	0.267
D	1.119	1.146	0.265	0.279	0.489	0.466	0.155	0.169	0.497	0.323	0.186	0.222
E	0.946	0.929	0.265	0.240	0.281	0.459	0.161	0.331	0.352	0.349	0.210	0.239
F	0.952	0.838	0.195	0.216	0.461	muFc 0.900	0.179	0.759	0.773	0.533	0.193	0.220
G	0.729	0.626	0.161	0.195	0.441	e' 0.850	0.173	0.403	0.482	0.897	0.191	0.223
H	0.560	0.472	0.187	0.483	0.480	0.957	0.175	0.177	0.376	0.754	0.235	0.407

To Page No.—

Witnessed & Understood by me,	Date	Invented by	Date
Irving Kong	11-28-97	Recorded by Jie chen	11/19/97

Project No.

Book No. 93013 TITLE

From Page No.

11/20/97

一. Sequencing Reaction

hulks in TA #1, 4, 5, 7

primer : 1019S, 1020A

二. Read sequencing Gel

clone # 2, 3 of hulks in TA are wrong.

三. minipre

TC3 (NCS^{2-}) in TA #1-10

cut with EcoR. I

#2, 3, 4, 5, 8, 9 are correct.

To Page N

Witnessed & Understood by me,	Dat	Inv nt d by	Date
Irving Kong	11-28-97	R c rd d by Tu chen	11/20/97

11/21/97.

-. Read sequencing film.

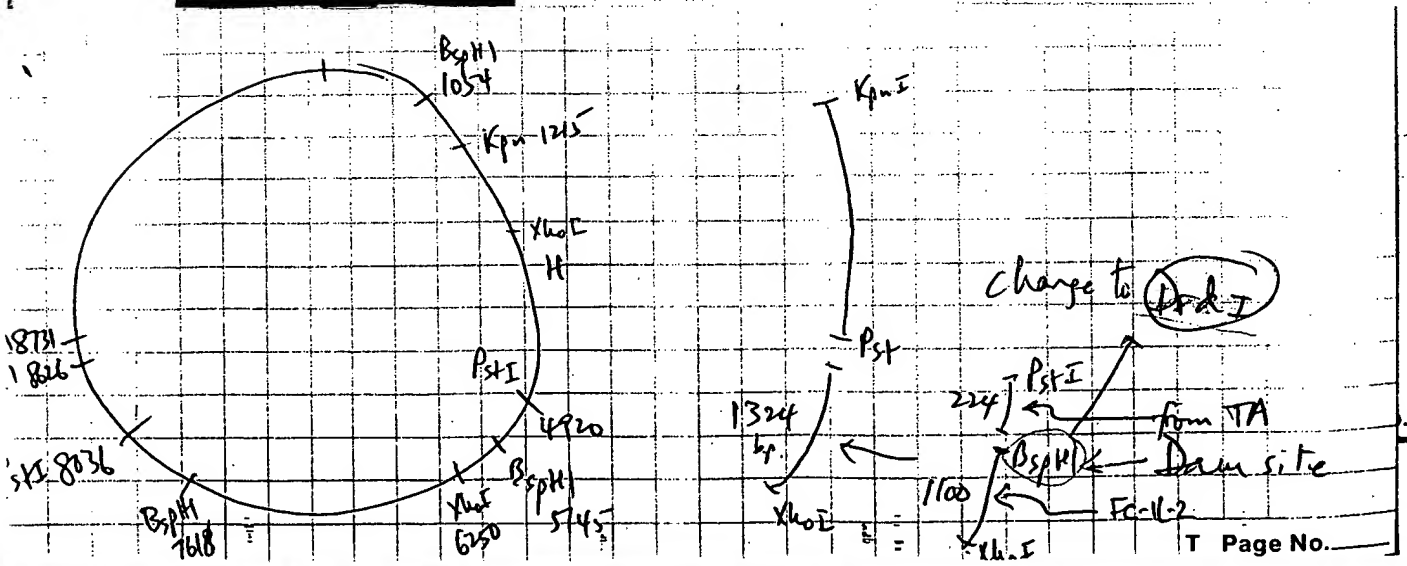
clone #5 of huks in TA is completely correct.

=. prepare fragment.

1. Vector : PstI/XhoI in SK

2. fragment : PstI/BsphI from huks in TA #5

BsphI/XhoI from huks-IL2



From Page No.

12/2/97

- prepare fragment

1. fragment : DrdI/PstI from hules in TA #5 (~285bp)

← 285bp (DrdI/PstI)

2. fragment : DrdI/XhoI from pHHg-hu14.18-IL2 (~1kb)

cut with DrdI

← DrdI/XhoI

= Ligation

Vector PstI/XhoI in BSX

insert PstI/DrdI (285bp)

DrdI/XhoI (1kb)

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
Sung Kong	12-28-97	Recorded by Jie chen	12/2/97

From Page No.__

12/3/97

-. Sequencing Reaction

γ4 in ks (SphI → HindIII Links → XhoI)

#4, 6

primer : 60b u

=. minipre

γ4 in Bsx (PstI - PvdI - XhoI) #1-12

cut with PstI / XhoI

there is no correct clone.

≡. Isolate Genomic DNA

1. MCB ks-IC2 N4-627 cultured (with MTX)
2. MCB 4.18-IC2 N58-12-17 cultured (with MTX)

T Page No.____

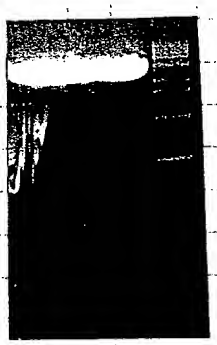
Witnessed & Understood by me,	Date	Invented by	Dat
Shunip Kong	12-28-97	Rec rded by Jia chen	12/3/97

From Page No.

12/4/97

- prepare frigment

vector ks / SmaI - XhoI



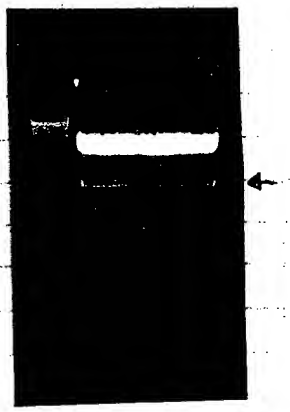
= Ligation :

repeat - 1. Construction of CD4 FcR4 (SphI → HindIII linker - XhoI) in ks

2. Construction of FcR4-X (PstI → AflII linker - XhoI) in ks

= Transformation.

1. Construction of CD4FcR4
2. construction of FcR4-X
3. R4 in BSX (PstI - HindIII - XhoI)



(D. Isolate genomic DNA

hucks - IL2 (N4-627) (~~elut~~ Cultured w/o MTX)

hu1.4-18 - IL2 (N58-10-17) (cultured w/o MTX + P/S)

To Page No.

Witnessed & Understood by m ,	Date	Invented by	Date
Kong	12-28	Recorded by Jie chen	12/4/97

From Page No.__

12/8/97

— Mini pre

γ4 in BSX (PstI - PrdI - XhoI) #1-14

1. Cut with PstI/XhoI

#3, 6 are positive.

2. take #3 6 to cut with PrdI.

This digestion is not completed. but we can see the insert of 1426bp. It means that there is a prdI site between xhoI ~~site~~ and pstI.

So #3 6 are correct.

To Pag N .____

Witnessed & Understood by me,	Date	Invented by	Date
Irving King	12-8	Record d by Jia chen	12/8/97

Express Mail Label No. EV260610557US

PATENT
Atty. Docket No. LEX-003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Gillies *et al.*	CONFIRMATION NO.:	9492
SERIAL NUMBER:	09/256,156	GROUP ART UNIT:	1647
FILING DATE:	February 24, 1999	EXAMINER:	Kapust, R.B.
TITLE:	Enhancing the Circulating Half Life of Antibody-Based Fusion Proteins		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF STEPHEN GILLIES, KIN-MING LO, YAN LAN, AND JOHN WESOLOWSKI UNDER 37 C.F.R. 1.131

Sir:

We, Stephen Gillies, Kin-Ming Lo, Yan Lan, and John Wesolowski, hereby declare as follows:

1. We are co-inventors of the subject matter described and claimed in the above-identified patent application.

2. We have reviewed claim 27 as submitted herewith. We make this declaration to establish invention of the subject matter of claim 27 prior to the effective date of International Publication No. WO 97/43316, entitled "Physiologically Active Molecules with Extended Half-Lives and Methods of Using Same," which allegedly published on November 20, 1997.

3. Prior to November 20, 1997, we conceived of and reduced to practice in the United States an antibody-based fusion protein for administration to a mammal, the fusion protein comprising a variable domain and a portion of an IgG4 CH2 domain, the C-terminus of which was linked by a CH3 domain to the N-terminus of IL-2. The antibody-based fusion

protein had a longer circulating half-life *in vivo* than an antibody-based fusion protein comprising a portion of an IgG1 CH2 domain linked to IL-2.

4. We attach as Exhibit A true copies of laboratory notebook pages 137 and 191 of Kin-Ming Lo, signed by Kin-Ming Lo, except that their dates, which are all prior to November 20, 1997, have been removed. These pages are evidence demonstrating that we conceived of and reduced to practice the invention of claim 27 in the United States prior to November 20, 1997.

5. Notebook page 137 shows a schematic representation of an expression vector that was prepared (see below the heading "KSγ_4-IL-2"). The vector included an Xho I-Hind III fragment encoding heavy and light chain variable regions derived from the mouse antibody KS-1/4; a Hind III-Nsi I fragment encoding the Cγ_4 region; and a Nsi I-Xho I fragment encoding IL-2. The nucleic acid fragments encoding Cγ_4 and IL-2 were oriented such that, upon expression of the protein, the C-terminus of the Cγ_4 heavy chain is linked to the N-terminus of IL-2.

6. As shown in the table at the bottom of Notebook page 191, experiments were performed using purified KS-1/4-IL-2 fusion proteins containing either Cγ_1 or Cγ_4 heavy chains to compare the *in vivo* circulating half-lives of these fusion proteins. The KSγ_4-IL-2 fusion protein was expressed from the vector described on Notebook page 137. The data in the table represent the concentrations of KSγ_1-IL-2 and KSγ_4-IL-2 measured in blood samples collected from mice at various time points after injection of the respective fusion proteins. KSγ_4-IL-2 had a longer circulating half-life *in vivo* than KSγ_1-IL-2.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like, so made,

are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: January 12, 2004 Stephen Gillies

Stephen Gillies

Date: Jan 12, 2004 Kin-Ming Lo

Kin-Ming Lo

Date: Jan. 12, 2004 Yan Lan

Yan Lan

Date: Jan 12, 2004 John Wesolowski

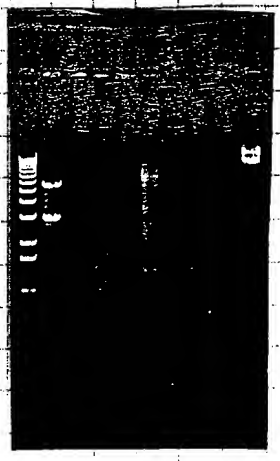
John Wesolowski

3003004

rom Pag No.

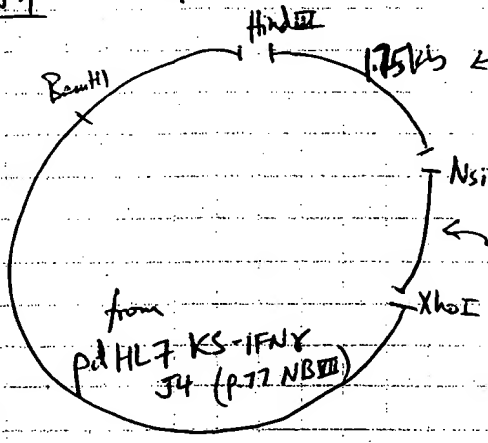
Jie sequenced the pdHL2-OKT3 w/ HD6
Sequence was very clean and it showed the STOP codon followed by the 3'-untranslated reg. of the γ1.
The size of the OKT3 also looks normal (see p.58 NB VIII)

Sumin cut OKT3 (the pdHL2 OKT3 maxi th. that was sequenced & used to transfect cells) and also pdHL2 OKγ4-9227 w/ the following enzymes. The NsiI frag @ 2.8 kb increased to ~3.6, showing the presence of the 9227 SCA.



Jie did the following:

γ4 KSγ4-IL-2



← from pdHL7 OKT γ4 9227 (γ4 and γ1 both have HindIII - NsiI frag of ~1.75 kb.)

← IL-2 from cmv-Fc-IL-2

Jie digested minipreps w/ HindIII + XhoI. #12 dropped out ~2.2 and 8 kb
Jie then digested #12 w/ XbaI 5 + 5.3
EcoRI : 2.7 + ~7.5
BamHI
FspI : 0 0 γ4 has FspI ~750 downstream of HindIII
Note XbaI digest of pdHL7 OKT γ4 9227 gave 2 kb + ~8 kb.

To Pag No. 191

Witnessed & Understo d by me, Jie Chen
Dat
Invent d by
Date
R c rded by K. Ng

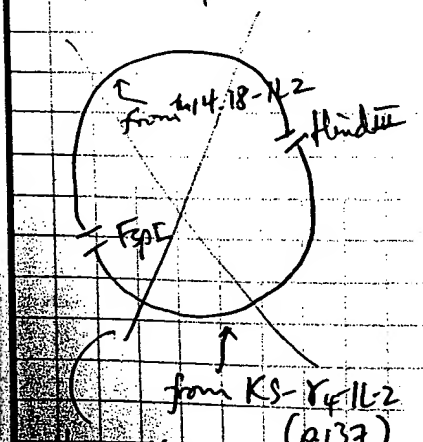
From Page No. 137

You did PK studies comparing KS-IL2 vs KS γ4-IL-2 (For construction, See p.137.)

Conclusion: KS γ4-IL-2 has a much longer half-life and the γ4-IL-2 is intact.

You digested the KS γ1-IL-2 KS γ4-IL-2 proteins O/N w/ plasmin.

SM will make the 14.18-γ4-IL-2



No, can't [illegible]

Hu Fc ELISA — H-Fc ELISA 3.0 mg/L

Mouse #	30'	1h	2h	4h	8h	24h	48h	72h
1	10.5	13	9		5.4	4.1		
KS-γ4-IL2 2	12.6	9	9		5.5	4.1		
3	12.0	7.6		6.4		5.2		
4	19.5	12.4		7.7		2.0		
(Mean)	13.6	10.5	9	7.1	5.4	3.9		
5	4.3	2.1	1.2		1.0	0.2		
6	4.4	2.4	1.9		1.0	0.5		
KS-γ1-IL2 7	4.3	2.0		1.1		0.5		
8	4.9	2.4		1.7		0.5		
(Mean)	4.5	2.2	1.5	1.4	1.0	0.43		

starting Material: KS-γ4-IL2 108 mg/L 103 mg/L
KS-γ1-IL2 121 mg/L 100 mg/L

Hu H&L / Hu IL2 ELISA — H-IL2 1.86 mg/L

Mouse #	30'	1h	2h	4h	8h	24h	48h	72h
1	9.2	10.3	8.2		5.5	3.3		
KS-γ4-IL2 2	11.7	8.4	6.8		4.6	3.3		
3	9.3	6.3		6.0		5.0		
4	(22.4)	10.2		7.6		1.5		
(Mean)	10.1	8.8	7.5	6.8	5.1	3.3		
5	3.6	1.4	0.65		0.65	0		
KS-γ1-IL2 6	3.4	1.3	1.0		0.60	0.05		
7	3.0	1.2		0.7		0.09		
8	3.6	1.3		0.9		0.1		
(Mean)	3.4	1.3	0.83	0.8	0.63	0.06		

starting Material: KS-γ4-IL2 124 mg/L 113 mg/L

To Pag No. 192

Witnessed & Understood by me, | Date | Invented by | Date

R corded by